

The University of Chicago

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OF ATABRINE

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY
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THE ACCUMULATION AND EXCRETION OF ATABRINE¹

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Received for publication March 12, 1943

The widespread use of atabrine points clearly to the need for a more thorough study of its pharmacologic and toxicologic behavior. Quantitative data concerning the rate and extent of accumulation of atabrine in experimental animals after repeated dosage, and the influence of diet and disease on the detoxication rate, are essential to the proper use of the compound in the chemotherapy of malaria.

Atabrine is generally considered to be a cumulative drug though the evidence for this is rather indirect. The low blood concentrations (1-5) together with the small amounts found in the urine and feces (2-4, 6-12) indicate that atabrine is rapidly absorbed and taken up from the blood by the tissues. The atabrine content of tissues has been studied (5, 13) after a single relatively large dose but the accumulation in the tissues after long continued administration of small doses has not been investigated. The slow fall in concentration of atabrine in the tissues (3) together with the observation that it is still detectable in the urine and feces 69 or 70 days after the cessation of treatment (11) suggests that if it were given on a long term basis, accumulation would occur. Hecht (6) and Martin, Cominole and Clark (14) observed non-characteristic pathological changes in animals following large oral doses of atabrine. With small doses, observable cumulative effects are absent or only slight over a period of 6 to 8 weeks (14).

The yellow pigmentation frequently seen after atabrine administration has been considered the most important clinical evidence of accumulation. It appears early in the course of treatment and may persist for as long as eighteen weeks after cessation of treatment (15). In animal experiments this pigment has been shown to be fluorescent (6) and is undoubtedly either atabrine or a derivative. Other symptoms suggestive of accumulation are headache, mental depression and psychoses.

The general acceptance of progressive accumulation in the tissues is probably responsible for such recommendations as those of Dawson, Gingrich and Hollar (16), "It seems reasonable that as a general rule, to lessen the risk of toxic effects from cumulation, atabrine treatment should not be repeated within eight weeks

¹ This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. It was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

² These studies were submitted to the University of Chicago in partial fulfillment of the requirements for the degree of Doctor of Philosophy, December, 1942.

of a previous course, and that the drug should be dispensed only on prescription of a physician."

Our studies were undertaken with a view to determining in dogs the amount of atabrine excreted in the urine and feces and the amount of accumulation in the tissues after small daily oral doses, comparable to those used in the suppressive treatment of malaria. The accumulation in the tissues was also determined after large daily oral doses and after simultaneous administration of atabrine and sulfonamide drugs.

METHODS. In all experiments the dogs were fed at the same time each day and atabrine dihydrochloride given orally one half hour later. Dosage levels of 5 and 50 mgm./kgm. were used. Unpublished work in this laboratory has shown that doses of 50 mgm./kgm. induce vomiting in dogs in 70% of the cases when given in gelatin capsules and in 96% of the cases when given as a water solution by stomach tube. To avoid this undesirable reaction the drug was given in the form of tablets coated with cellulose-acetate-phthalate, a new enteric coating supplied by Eastman Kodak Company. The smaller doses were given in gelatin capsules. Feces and urine collections were made daily just before feeding. All analyses and doses are expressed in terms of atabrine dihydrochloride.

Blood level and urinary and fecal excretion studies were made on four dogs which were kept in individual metal metabolism cages; the urine and feces were collected daily just before the administration of atabrine. Blood samples were taken daily just before giving atabrine during the first week, and at 24 and 48 hour intervals after the last dose of each weekly series thereafter. Estimations of the concentration of atabrine in the blood of these dogs were made by direct iso-amyl alcohol extraction of laked whole blood buffered to pH 8.5 and determination of the fluorescence of the alcohol extract. During the first week of the experiment, atabrine in the urine and feces was determined daily. After this time aliquots of the daily collections were pooled and stored in the refrigerator until the end of the week when determinations of the weekly excretion were made. The feces of the animals receiving 50 mgm./kgm. were analyzed for atabrine to determine the absorption from the enteric coated tablets. Control experiments showed no change in the atabrine content on storage in the refrigerator.

The distribution in the tissues was studied in 24 dogs. In all experiments the dogs were sacrificed 48 hours after the last dose. Two dogs were analyzed after a single dose of 5 mgm./kgm. and two each after one, two, three and four weeks courses of atabrine 5 mgm./kgm. per day for six days each week. Two dogs which had received approximately 30 mgm./kgm. per week for eight weeks followed by 15 mgm./kgm. twice each week for four and one half weeks were given 5 mgm./kgm. per day for three days and then analyzed. Two dogs were sacrificed after seven and two after fourteen daily doses of 50 mgm./kgm. Four dogs were given 50 mgm./kgm. of sulfathiazole three times a day simultaneously with the third week of atabrine (5 mgm./kgm. per day, six days a week), and four dogs were given 50 mgm./kgm. of sulfadiazine three times a day simultaneously with the fourth week of atabrine (5 mgm./kgm. per day). Free and total blood sulfathiazole or sulfadiazine were determined on these eight dogs and also on four controls for each sulfonamide.

The concentration of atabrine was determined in the adrenals, bile, blood, bone marrow, brain, heart, kidney, liver, lung, muscle, pancreas, skin and spleen. Analyses were done in duplicate except for the adrenals, bile and bone marrow. The deviation of the duplicates from the average was less than 5%.

Method of Atabrine Analysis: The sample was blended with at least four parts by weight of water, and 25 gram aliquots transferred by pipette to tared 100 cc. beakers. After weighing, 25 cc. of 2N HCl was added, and the samples digested by warming on a steam bath for two hours. The volume was maintained by addition of distilled water.

After the digestion period, the sample was cooled in the refrigerator and saturated NaOH added to make the mixture alkaline. The sample was immediately transferred to a separatory funnel and an alkaloid extract prepared exactly as already described (17) for the determination of quinine. One cc. of concentrated NH_4OH was added to the extract

in a 50 cc. volumetric flask and water added to the mark. For the fluorometric assay the Coleman Model 12 Electronic Photofluorometer was used with B-1 and PC-1 filters, with quinine as a reference standard.⁴ This method was primarily designed for tissue and excretion studies. The concentration of atabrine in the blood is too low for accurate determination by this method except when very large doses of atabrine are used.

A dog which had never received atabrine was sacrificed, and the various tissues analyzed for "apparent atabrine" concentration. Most tissues were found to have a small amount of extractable fluorescent material. This was especially noticeable in the feces, but even here the concentration was 1 mgm./kgm. or less. This amount was considerably below the

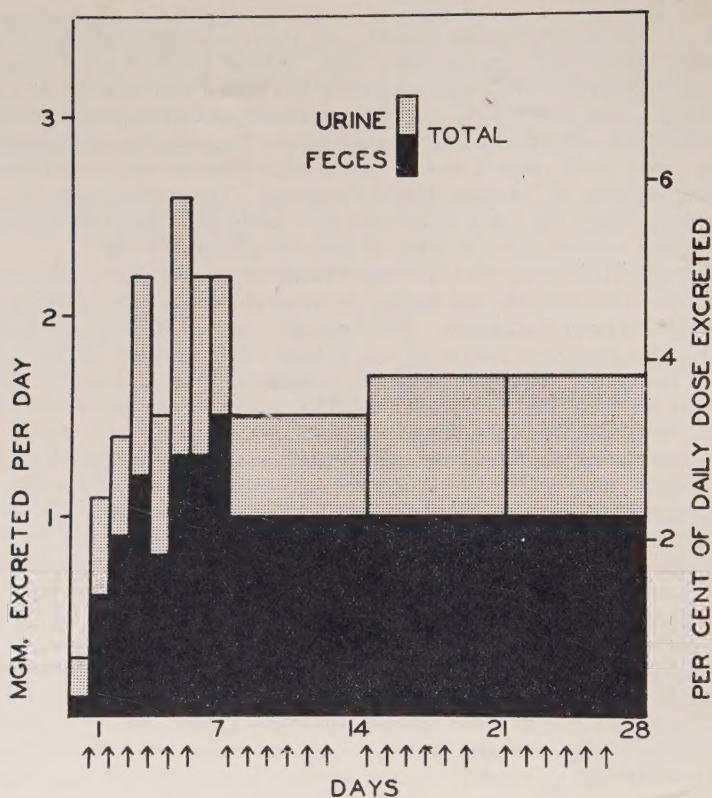


FIG. 1. EXCRETION OF ATABRINE IN THE URINE AND FECES
Arrows designate days on which atabrine was given

concentration observed in experiments with atabrine. The recovery of known amounts of atabrine added to tissues ranged from 92 to 113%. Recovery from aqueous solutions of pure atabrine was always within 10%.

RESULTS. Figure 1 shows the daily excretion of atabrine in the urine and feces for one week and the average daily excretion for each of the three succeed-

⁴ Later studies on the sensitivity and specificity of this method have led to two improvements. The results are more reliable when the alkaloid extract is made up in a final concentration of 50% alcohol with 1 cc. of concentrated NH_4OH added for each 50 cc., and more nearly specific for atabrine when the B_2 PC-2 filter combination is used. Certain atabrine derivatives have been found to fluoresce more strongly to the 365 mm. Hg. line (B_1) than to the 436 mm line (B_2), and the resultant fluorescence of these derivatives passes the PC-1 filter more readily than the PC-2.

TABLE 1
Distribution of atabrine in tissues

TISSUE	DOSAGE LEVEL										
	50 mg./kg.			5 mg./kg.							
	Number of daily doses										
	0	7	14	1	6	12	18	24	78*	18†	24‡
	Concentration of atabrine dihydrochloride in mg./kg. of tissue										
Adrenals	0.1	198	445	7.3	18.3	31.2	22.5	26.7	12.7	3.1	15.8
		63	199	3.3	10.7	20.1	15.6	13.3	15.4	15.4	18.9
										3.9	2.1
										5.6	8.2
Bile	0.1	207	310	1.9	9.5	2.3	2.4	8.8	6.7	1.3	6.7
		23.4	208	3.4	22.4	7.9	10.7	14.9	10.4	3.4	2.8
										2.4	1.4
										10.5	2.6
Blood	0.0	1.0	1.5	0.0	0.2	0.1	0.0	0.3	0.1	0.0	0.0
		0.3	2.8	0.0	0.1	0.0	0.3	0.0	0.0	0.0	0.0
										0.0	0.0
										0.0	0.0
Bone marrow		88.0	109	0.8	14.7	5.7	5.0	1.9	5.9	1.2	7.4
		16.5	107	0.0	6.6	2.4	15.2	1.5	8.1	4.3	2.2
										2.3	0.5
										3.9	2.4
Brain	0.3	7.9	9.8	0.4	1.5	1.9	0.7	1.0	0.7	0.1	0.9
		1.9	5.9	0.8	0.8	1.1	0.8	0.3	0.9	0.3	0.7
										0.2	0.2
										0.4	0.3
Heart		60	91	1.8	5.6	6.4	4.6	5.4	1.6	0.1	11.2
		10	48	1.0	2.8	4.0	4.9	2.4	3.7	1.1	2.6
										1.2	0.8
										0.6	1.1
Kidney	0.0	49	90	1.8	7.8	5.5	3.9	11.5	5.0	1.3	11.7
		16	88	1.0	2.8	4.0	4.9	2.4	3.7	3.3	10.3
										2.6	1.6
										1.5	2.9
Liver	0.2	737	995	8.0	28.5	40.0	21.0	17.0	12.2	2.9	19.0
		59	705	5.7	33.8	29.0	25.9	10.0	35.2	12.8	25.0
										5.9	6.8
										4.0	6.6
Lung	0.1	77	166	2.0	16.0	13.6	11.2	26.7	13.0	3.6	22.0
		50	154	2.0	8.9	14.8	11.4	8.0	19.5	0.1	15.6
										6.7	8.1
										2.2	7.4

* These dogs received the equivalent of 78 doses over a period of 13 weeks.

† These dogs received in addition 50 mg./kg. of sulfathiazole t.i.d. the last 6 days.

‡ These dogs received in addition 50 mg./kg. of sulfadiazine t.i.d. the last 7 days.

TABLE 1—*Continued*

TISSUE	DOSAGE LEVEL										
	50 mg./kg.			5 mg./kg.							
	Number of daily doses										
	0	7	14	1	6	12	18	24	78*	18†	24‡
	Concentration of atabrine dihydrochloride in mg./kg. of tissue										
Muscle		13	21	0.6	3.2	1.9	0.5	1.5	0.7	0.1	5.8
		10	22	0.1	1.6	1.2	2.8	0.5	1.1	0.4	0.6
										3.5	0.1
										4.3	0.2
Pancreas	0.1	120	372	7.8	42.0	33.0	22.5	48.8	43.6	3.6	5.7
		62	242	3.7	23.5	30.0	50.0	21.8	63.5	18.1	27.2
										6.3	7.2
										15.4	19.0
Skin		16	51			1.4	0.8	5.5	4.2	1.5	2.8
		10	15	1.0	1.3	1.1	2.1	2.6	5.4	1.3	1.5
										1.0	1.6
										0.4	4.4
Spleen	0.1	168	232	5.2	42.0	22.0	11.6	32.4	21.2	2.8	22.2
		71	263	4.4	16.5	42.5	30.2	15.0	50.0	25.6	21.2
										8.4	8.4
										6.6	2.2

ing weeks. In all cases the per cent of the daily dose excreted in the urine and feces was less than six although a considerable amount of fluorescent atabrine derivatives, not extracted by this method, was present in the urine. Stabilization of the per cent of the daily dose excreted was attained within two weeks.

At the time of these experiments, no satisfactory method was available for the determination of low blood levels of atabrine. The fluorescence of the iso-amyl alcohol extract of the laked blood gave an indication of the amount of total fluorescent alcohol-soluble material but it was not considered sufficiently sensitive or specific to warrant presentation of the individual data obtained. However, in no instance did the concentration of extracted fluorescent material (calculated as atabrine dihydrochloride) rise above 1 mgm./l., and the average level was about 0.5 mgm./l. These observations are in agreement with those of other investigators (1-5) and serve only to emphasize the rapid removal of atabrine from the blood.

In the dogs receiving 50 mgm./kgm. per day as enteric coated tablets no vomiting occurred. The animals showed some weight loss and a severe diarrhea, but in spite of the diarrhea, fecal analyses showed that approximately 80% of the administered drug had been absorbed. The high concentrations attained in the tissues are further evidence of the completeness of absorption.

The distribution of atabrine in the tissues is shown in table 1. Although the

spread of values obtained from each pair of dogs on the 5 mgm./kgm. dosage level was wide, in general the maximal concentrations were attained in one week. The adrenals, heart and liver gave slightly higher values for the second week. No tissue showed a significantly higher concentration at 13 weeks with the exception of the skin and possibly the pancreas. The liver showed a significant drop in the two to four week period. The adrenals, kidney, bile, bone marrow, heart, muscle and brain showed equivalent or slightly lower values at thirteen weeks. The tissue concentrations attained with the 50 mgm./kgm. dose were approximately ten times those found after 5 mgm./kgm. dosage.

Since after the first or second week of daily atabrine administration, neither the elimination nor the storage of the drug is increased, and since an average of less than 4% is eliminated in the urine and feces, the dog must convert approximately 96% of the daily intake to other substances. Unpublished data from this laboratory show that there is no histologically detectable damage to the liver after eight weeks and no apparent effect on liver function (by bromsulphalein test) or the general health of the dogs after 32 weeks during which they received 30 mgm./kgm. per week.

In the dogs which received sulfathiazole simultaneously with atabrine the tissue levels attained were considerably lower than when atabrine was given alone for an equal period of time. With sulfadiazine the deviations from the controls were not so marked but the trend was in the same direction as with sulfathiazole. The atabrine had no effect on the blood levels of either free or total sulfathiazole or sulfadiazine.

SUMMARY

The excretion of atabrine in the urine and feces was studied throughout a period of four weeks of daily atabrine administration. The average daily excretion soon reached a level of less than 4% of the daily dose.

Determinations of the atabrine concentration in various tissues of the dog 48 hours after the last of a series of small daily oral doses showed the maximum concentrations in most tissues to be reached within two weeks.

Higher tissue levels are attained with larger doses, those reached with 50 mgm./kgm. being about ten times those reached with 5 mgm./kgm.

Cellulose-acetate-phthalate is a satisfactory enteric coating for administering relatively large doses of atabrine since no vomiting occurs and about 80% of the administered drug is absorbed.

The administration of atabrine with either sulfathiazole or sulfadiazine did not affect the blood levels of the sulfonamides, but the concentration of atabrine in the tissues was lowered.

REFERENCES

- (1) CHOPRA, R. N., S. K. GANGULI AND A. C. ROY, *Ind. Med. Gaz.*, **71**: 443, 1936.
- (2) FARINAUD, E., C. LATASTE, J. BACCIALONE AND J. CANET, *Compt. rend. Soc. de biol.*, **130**: 623, 1939.
- (3) LATASTE, C., M. E. FARINAUD AND NGUYEN-VAN-LIEN, *Ibid.*, **130**: 522, 1939.
- (4) FARINAUD, E., C. LATASTE, J. BACCIALONE AND J. CANET, *Bull. Soc. path. exot.*, **32**: 513, 1939.

- (5) DIKSHIT, B. B., Report Haffkine Inst. for the year 1938, p. 47.
- (6) HECHT, G., Arch. f. exper. Path. u. Pharmacol., **170**: 328, 1933.
- (7) TROPP, C., AND W. WEISE, Ibid., **170**: 339, 1933.
- (8) WEISE, W., Acta tertii de trop. atque malar. morbis, **2**: 489, 1938.
- (9) KEHAR, N. D., Rev. Malar. Surv. India, **5**: 393, 1935.
- (10) ASHBEL, S. I., J. Physiol. (U.S.S.R.), **25**: 171, 1938.
- (11) MASSA, M., Riforma med., **49**: 980, 1933.
- (12) RONCORONI, C., Gior. di clin. med., **16**: 1370, 1935.
- (13) HECHT, G., Arch. f. exper. Path. u. Pharmacol., **183**: 87, 1936.
- (14) MARTIN, S. J., B. COMINOLE AND B. B. CLARK, THIS JOURNAL, **65**: 156, 1939.
- (15) SCHECHTER, S. J., AND H. H. TAYLOR, Am. J. M. Sci., **192**: 645, 1936.
- (16) DAWSON, W. T., W. GINGRICH AND E. D. HOLLAR, Am. J. Trop. Med., **15**: 515, 1935.
- (17) KELSEY, F. E., AND E. M. K. GEILING, THIS JOURNAL, **75**: 183, 1942.

